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Studies on long term behavioural changes in group-housed rat models of brain and spinal cord injury using an automated home cage recording system

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Abstract:

Background: Neurotrauma patients face major neurological sequelae. The failure in the preclinical-to-clinical translation of candidate therapies could be due to poor evaluation of rodent behaviours after neurotrauma.

New Method: A home cage automated system was used to study the long term behaviour of individual rats with traumatic brain injury (TBI), spinal cord injury (SCI) and non-CNS injured controls, whilst group-housed in their home cages. Naïve rats were used as baseline controls. Automated locomotor activity and body temperature recordings were carried out 24 h /day for 3 days/week during 12 weeks post-injury. Behavioural patterns, including aggression, rearing, grooming, feeding and drinking were analysed from automated video recordings during week 1, 6 and 12.

Results: SCI animals showed a lower locomotor activity compared to TBI or control animals during light and dark phases. TBI animals showed a higher aggression during the dark phase in the first week post-injury compared to SCI or control animals. Individual grooming and rearing were reduced in SCI animals compared to TBI and control animals in the first week post-injury during the dark phase. No differences in drinking or feeding were detected between groups. Locomotor activity did not differ between naïve male and female rats, but body temperature differ between light and dark phases for both.

Standard methods: Injury severity was compared to standard SCI and TBI behaviour scores (BBB and mNSS, respectively) and histological analysis.

Conclusions: This study demonstrates the practical benefits of using a non-intrusive automated home cage recording system to observe long term individual behaviour of group-housed SCI and TBI rats.

Introduction

Traumatic injuries are the single greatest cause of lost human potential worldwide, and traumatic brain injury (TBI) and spinal cord injury (SCI) are associated with death or lifelong disability (1). Furthermore, their incidence is increasing, due to the global aging of the population.

TBI (2) and SCI (3) involve two distinct phases of injury – the primary injury caused immediately by the mechanical insult, and the secondary injury, evolving over time through a cascade of vascular, cellular and biochemical events (4). Despite advances in pre-hospital trauma management, there are no effective treatments to reverse the primary CNS damage and most therapeutic developments focus on modulating the progressive secondary injury, to support regeneration of the injured CNS.

Despite a large number of preclinical studies, generally with apparent robust validity, treatments have shown very limited impact in the clinic. Yet, research on CNS injury must advance and *in vivo* modelling still remains an instrumental tool for mechanistic studies on injury pathophysiology (5).

Assessment of functional impairment remains critical for CNS modelling in which motor and/or sensory recovery tests are often used. The BBB locomotor scale is a standard kinematic measure used to assess hindlimb motor recovery following thoracic SCI in rats

43 (6). Other tests such as the grip strength test, which measures muscle strength (7), or the
44 Hargreaves hot plate or von Frey filaments tests, can also be used in SCI models to
45 assess thermal hyperalgesia and mechanical allodynia, respectively (7, 8). The modified
46 neurological severity score (mNSS) is commonly used in rodent models of TBI to evaluate
47 motor, sensory, proprioceptive and reflex behaviours (9, 10).

48 However, these behavioural tests are biased towards assessing task driven and not
49 spontaneous behaviour, which may poorly reflect translatable outcomes with therapeutic
50 impact (11). Most studies implement test batteries which have many confounders, such
51 as test time and order, environment enrichment and acclimatization time (12).
52 Furthermore, most behavioural tests involve momentarily removing the animal from its
53 home-cage and social group and exposure to a new and unfamiliar environment (13, 14)
54 which is then confounded further but the impact of different handlers and handling
55 expertise. Also, many of these tests only allow for a “snap-shot” assessment of daily
56 behaviour, missing infrequent disease phenotypes that happen outside a window of
57 observation (e.g. seizures at night) (15). Moreover, rodents are crepuscular (16, 17), so
58 solely assessing them during the working day of a research scientist will very likely mask
59 the full extent of relevant neurobehavioural changes. Thus, classical assessment of
60 rodent behaviour needs to be complemented with other unforced and non-stimulated
61 automated assessment approaches in the home cage over long time intervals. This is
62 particularly relevant to investigate the impact of injury on cognitive and social functions
63 and the potential therapeutic benefits in neurotrauma models.

64 Body temperature, which is infrequently studied can be a valuable indicator of
65 homeostasis during surgery and post-operative care, could directly impact recovery from

66 CNS injury. Furthermore, a large variability in body temperature, due to the inflammatory,
67 cardiovascular and/or shock response, could impact drug testing outcomes (18).
68 Therefore, regular monitoring across the light and dark phases is critical in studies
69 involving neurotrauma models.

70 Recently, one technology available to researchers is the home cage analysis (HCA),
71 which facilitates the assessment of caged animals in their undisturbed 'home'
72 environment. HCA systems utilise a variety of technological modalities, including video
73 technology, infrared (IR) sensors and telemetry (17). Most systems rely on one-or-two of
74 these approaches, and have been successfully used to characterise individual
75 behavioural profiles in rodent models of Huntington's disease and prion diseases (17),
76 and also some studies have been reported in single housed neurotrauma mouse models(
77 REF-Ping). Few systems support long-term monitoring and data analysis on grouped
78 housed animals.

79 Recently, an automated home cage recording system was developed by Actual Analytics
80 Limited in collaboration with the National Centre for the 3Rs (NC3Rs), which was capable
81 of capturing individual temperature and behavioural data of rodents group-housed in
82 normal home cages over long periods of time (12, 19).

83 To investigate the utility of this automated home cage recording system in traumatic CNS
84 injury, we used this recording system to monitor changes in the behavioural phenotype
85 of group-housed rat models of TBI and SCI, during sub-acute and chronic post-injury
86 phases. Automated body temperature and basic behavioural monitoring was completed
87 using non-invasive, automated telemetry and digital data collection throughout both light
88 and dark phases for up to 12 weeks post-injury. Subsequent manual review of

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<https://www.ncbi.nlm.nih.gov/pubmed/30176241>

Vu et al (2018) Transient disruption of mouse home cage activities and assessment of orexin immunoreactivity following concussive- or blast-induced brain injury

This study uses the Any-Maze cage (AMc) housing and activity monitoring, which is for a single mouse

<https://www.ncbi.nlm.nih.gov/pubmed/27073377>

* Qu (2016) Automated monitoring of early neurobehavioral changes in mice following traumatic brain injury
SmartCage system is a non-invasive home cage rodent behaviour monitoring system, which is for a single mouse

corresponding IR video data was completed to derive more complex neurobehavioural insights.

Methods

Ethical statement

All animal procedures were carried out under two Project Licences (PPL 70/8712 and PPL 70/7436) approved by the Animal Welfare and Ethical Review Body at Queen Mary University of London and the UK Home Office, in accordance with the EU Directive 2010/63/EU. All animal facilities and suppliers have been approved by the UK Home Office Licensing Authority and meet all current regulations and standards for the UK. A total of 24 rats were used for the work described in the study, 18 of which underwent surgical recovery procedures. For this exploratory study we used $n=6$ animals per group, based on our previous efficacy studies using these neurotrauma models (20, 21) to provide a valuable discriminatory power of 80% with a significant level $\alpha = 0.05$ to detect approx. 20% relative differences in behaviour and histological assessments as primary outcomes for our neurotrauma studies. Experimental planning for data randomization and blinding data acquisition and analysis was carried out following the ARRIVE guidelines (22).

Animal housing and husbandry

A total of 24 adult Sprague-Dawley rats (weight range 200 - 300 g; 9 - 10 weeks old at the start of the study) were obtained from Charles River Laboratories, Margate, UK. Health screens provided by the official vendor indicated that rats were free of known

111 pathogens in accordance with FELASA Recommendations for health monitoring of rodent
112 colonies (23). Animals were housed in groups of 3 per Individually Ventilated Cage (IVC;
113 Allentown Europe, UK), in a 12 h light dark cycle (06:30 - 18:30 light; 18:30 - 06:30 dark),
114 with controlled room temperature (21 ± 1 °C) and relative humidity (40-60 %). The cages
115 contained 1-1.5 cm layer of animal bedding (Lignocel®, Rettenmaier UK Ltd). Rats had
116 access to food (Labdiet® EURodent 14% Diet 5LF2, LabDiet, Brentwood, Missouri, U.S.)
117 and water *ad libitum*. Rats were allocated to cages on arrival and remained in the same
118 social group throughout the study, including a 7 day acclimatization phase to the
119 laboratory.

120 ***SCI and TBI surgical procedures and in vivo experimental design***

121 Surgery was carried out in accordance with protocols reported previously (21, 24). All
122 animals were anaesthetised intraperitoneally with ketamine (Ketaset®) (50mg/kg) and
123 medetomidine (Domitor™) (0.2mg/kg), followed by subcutaneous administration of
124 buprenorphine (Buprenex®) (0.1mg/kg) for prophylactic analgesia. For TBI surgery, the
125 rat head was clipped, surgically scrubbed and subsequently secured to a stereotactic
126 frame using mouth, nose and ear bars, before a sagittal incision was made through the
127 scalp to expose the cranium. Utilising the PCI3000 Precision Cortical Impactor™
128 (Hatteras Instruments, Cary, NC), a “closed” TBI was induced by directly delivering a blunt
129 impact using a 5 mm diameter impactor tip to the right parietal bone, with the central
130 coordinates set at -3.5 mm from bregma and -3.5 mm from the midline. The impaction
131 was carried out using a 3.0 m/s velocity, a 3.0 mm impact depth, a 100 ms dwell time, at
132 a 20° angle to the bone. Following impact to the skull, the scalp was sutured, and animals
133 were placed in a warm incubator (27–28 °C) to recover. Reversal of anaesthesia involved

134 subcutaneous administration of atipamezole (Antisedan®) (0.1mg/kg). For SCI surgery,
135 the anaesthetised rats underwent a midline incision through thoracolumbar fascia on a
136 clipped and surgically scrubbed skin area, and the underlying muscles were pulled away
137 from the T9 – T11 spinous processes and laminae. The lateral aspects of the T9 and T11
138 vertebral bodies and spinous processes were clamped to stabilize any movement of the
139 spinal cord. A bilateral laminectomy was performed at T10, leaving the dura exposed but
140 intact. After securing the spinal column, the PCI3000 Precision Cortical Impactor™
141 (Hatteras Instruments, Cary, NC) was used with the following settings: a 2 mm impactor
142 tip, 1.5 m/s velocity, 1.8 mm impact depth, and 100ms dwell time, at a 90° angle to the
143 cord (24). Sham laminectomy animals underwent the same procedure as SCI-treated
144 animals, excluding the contusion injury on the spinal cord. Upon completion of spinal
145 surgery, the spinal fascia and muscle followed by the skin were sutured. Atipamezole was
146 administered, and the rat was placed in a warm incubator to recover (27 – 28 °C). Finally,
147 a radio frequency identity detection (RFID) chip was ‘injected’ subcutaneously into the
148 right flank of each rat, to permit tracking by the ActualHCA system. During the
149 postoperative recovery phase, all animals received buprenorphine (Buprenex®)
150 (0.1mg/kg) analgesia together with saline, subcutaneously administered twice daily for 3
151 days after surgery. Bladders were manually expressed twice a day for the SCI animals
152 until return of bladder function (<2 ml of urine in early morning expression for three
153 consecutive days).

154 The study was carried out in two consecutive periods of 12 weeks, for all experimental
155 groups (SCI, TBI and non-CNS injured control; randomly n=3 per group) to reach a total
156 of n=6 animals per group. Sex allocation was informed by the literature; female rats are

commonly used for SCI studies and male rats for TBI studies. To test for gender effect on locomotor activity and body temperature, 6 surgery-naïve control animals (n=3 males and n=3 females) were also used.

Conventional behaviour tests

Using the Basso, Beattie, Bresnahan (BBB) locomotor rating scale, open field locomotion assessment was carried out daily during the first week post-injury and then once weekly over 11 weeks, to characterize the functional outcome after spinal injury in the SCI and non-CNS injured control group (Suppl. Fig. 1A). A modified neurological severity score (mNSS) was used to evaluate motor ability, balance and alertness during the first week post-injury in the TBI group (Suppl. Fig. 1B).

Histology

At the end of the study (12 weeks post-injury) animals were deeply anaesthetized with sodium pentobarbital (50 mg/kg, i.p.; Sagatal, Rhone Merieux, Harlow, UK), and received a transcardiac perfusion with phosphate-buffered saline (PBS; 0.01 M, pH 7.4), followed by 4 % paraformaldehyde (PFA) in phosphate buffer (0.1 M, pH 7.4). Tissues were dissected out, post fixed in 4 % PFA for 2 h, and cryoprotected in 20 % sucrose in 0.1 M phosphate buffer at 4°C until further processing. Serial 20 µm coronal sections of whole brain and horizontal sections of spinal cord (extending approximately 1 cm rostral and 1 cm caudal from the contusion centre) were cut using a cryostat for histology. Representative serial sections were processed for Cresyl Violet (Nissl) staining. All brain tissue staining was performed between bregma - 1.28 mm and bregma -2.34 mm, where the lesion was located. Spinal cord staining was performed between the dorsal contusion site and approximately half the cord thickness.

Automated home cage recording system

The automated home cage recording system (Home Cage Analyser (ActualHCA™) system, Actual Analytics Ltd, UK) was used and specially fitted for standard IVCs (Allentown). Each automated home cage recording system was placed on a bespoke frame to support the placement of the IVC directly on top of the baseplate RFID reader. The infrared HD video camera and mini-computer for data recording were placed on a side slot frame facing one of the long sides of the IVC. On the top of the frame, an infrared lighting panel was placed above the top of the IVC (see Figure 1 for representation of the HCA system set up).

RFID transponders for animal identification and temperature measurements were supplied by BioMark (Boise, ID83702, US). The Biomark BioTherm13 Passive Integrated Transponder (PIT) is an RFID device with a 2.1 ± 0.1 mm diameter and 12.0 ± 0.4 mm length applied for subcutaneous implantation (ISO standards 11784/11785). All devices were factory calibrated (temperature range 33.0 - 43.0 °C). The baseplate RFID reader was designed to work with BioTherm13 RFID transponders.

The baseplate RFID reader consists of an array twelve transceiver coils, situated in waterproof casing underneath the cage. Each individual coil covers a separate 12 x 12cm square region underneath the cage floor and can detect the presence of an RFID chip up to a height of 13 cm. The twelve coils are arranged in a regular 3 x 4 grid spanning a total area of 36 x 48 cm, allowing motion in the plane of the cage floor (30 cm x 41 cm) to be recorded. Activity in the vertical plane (e.g. rearing) is not captured by the baseplate reader, but can be extracted from the concurrent video recorded (33). Rats are detected by the nearest antenna reading the ID and temperature from the RFID transponder.

203 Intermediate positions between adjacent antennae are sorted by applying a filtering
204 correction algorithm (33). When more than one animal is detected by the same antenna,
205 the greater strength signal corresponds to the closest animal (19). Continuous video
206 recordings were acquired by using infrared (IR) LEDs at 860 nm wavelength to illuminate
207 the cage from above, and USB 3.0 cameras with matched 4.5 mm lenses and daylight
208 filters (700 nm cut-off) were used to capture grayscale videos at 25 fps at HD (720p)
209 resolution.

210 **Data acquisition**

211 At a pre-defined time, animals were transferred into the automated home cage recording
212 system (Fig. 1). Three animals per experimental group (SCI, TBI, control) were studied
213 weekly for a 12 week study period, since the automated recording system functions
214 optimally when only tracking 3 animals within the same cage (25). For the CNS-injured
215 and non-CNS-injured control animals, RFID data (animal ID, locomotor activity and
216 temperature) and IR video data were captured 24 h/day, 3 days/week, for up to 12 weeks.
217 Naïve non-surgery animals (n=3 male in 1 cage; n=3 female in 1 cage) were studied for
218 5 days only.

219 Actual HCA Capture™ software (Actual Analytics Ltd, Edinburgh, UK) was used to
220 manage data capture and system calibrations, before IR video and matched baseplate
221 RFID data were stored to a local hard drive. Throughout each experiment, data analysis
222 was carried out using a time-binning of 5 min and video segment length of 30 min. Time-
223 binning indicates the duration of time represented by each datum in the data analysis
224 report (e.g. 100 mm travelled in 5 min).

225 **Data sampling and analysis**

226 RFID data were pooled and analysed using the Actual HCA Analyser™ software (Actual
227 Analytics Ltd, Edinburgh UK). We plotted 'transitions' against time as a measure of
228 'Locomotor Activity' (33). Specifically, one transition defines the movement of an animal's
229 RFID chip across the electromagnetic field boundary between two adjacent antennae.
230 This measure directly correlates with locomotion activity and distance. Subcutaneous
231 body temperature was also recorded via RFID chips.

232 Automatic RFID recordings aligned with the IR video recordings (~13.72 GB data per day
233 for a single cage of 3 animals in VLC media and HDF5 file formats) were used to visually
234 investigate selected behaviours (aggression, grooming, rearing, feeding and drinking).
235 With over 216 days (~23 days/week x 12 weeks x 6 groups = 216 days) of IR video
236 footage recorded, data sampling was required. RFID activity automatic data was tracked
237 per group per week 1, 6 and 12, to represent early subacute, late subacute and chronic
238 phases of injury, respectively (26, 27). Periods showing larger activity patterns were
239 selected for visualization of the video recorded data to better identify the display of
240 behavioural expressions (Suppl. Fig. S2). The five behaviours of interest were selected
241 based on their frequency of occurrence, after reviewing preliminary IR video footage and
242 ease of detection and insight into regular behaviour: aggression, grooming, rearing,
243 feeding and drinking. Further characterization and details are summarized in Fig. 2.

244

245 **Statistical analyses**

246 All the behaviour (the primary study endpoint), was assessed blind, with the researcher
247 unaware of the allocated intervention. Data from the two 12 week recording periods were

248 pooled together such that n=6 per experimental group, with n=3 animals grouped per
249 cage was analyzed.

250 Locomotor activity data were not normal distributed, so were analyzed using Kruskal-
251 Wallis Test (with Pairwise Mann-Whitney U test post hoc analysis tests). Temperature
252 data were normally distributed, so were analyzed using two-way ANOVA (with Tukey's
253 post hoc analysis tests). Data were shown as mean and standard error of the mean (SEM)
254 and comparisons were selected as statistically significant at $p < 0.05$. These analysis
255 were performed in R v3.5.1.

256 For the specific behavioural expressions (i.e. aggression, individual grooming, rearing,
257 feeding and drinking data acquired in combination from the RFID digital data with the IR
258 video recordings) mean \pm SEM were calculated for the duration of time and each
259 behaviour was expressed during a sample 5 min period per 12 h light or dark phase per
260 group per week (pgpw). Temporal changes in behavioural phenotype within each group,
261 and differences in phenotype between groups at defined time-points, were each assessed
262 by two-way ANOVA and Tukey's post-hoc test when statistical significance was identified.
263 Statistical significance was set at $p < 0.05$. These analyses were performed using Prism,
264 version 7.03 (GraphPad Software Inc., San Diego, CA).

265 A correlation analysis (Ping_which test?) was used to assess the association between the
266 information provided by the automated RFID recordings (Nm of transitions indicating
267 locomotor activity; including light and dark phase activity analysis) and the conventional
268 behaviour tests, and also the histological endpoints (spinal cord cavity and ventricle sizes
269 for the SCI and TBI groups, respectively). These analyses were performed using Prism,
270 version 7.03 (GraphPad Software Inc., San Diego, CA).

Results

Subacute behavioural analysis of naïve animals

Locomotor activity and temperature data demonstrating circadian pattern

Using the automated RFID digital data from the automated home cage recording system, locomotor activity and temperature data for naïve male and female rats were collected from 5 days (24/7 recordings; 3 rats/ group). Naïve animals showed no significant difference in locomotor activity and body temperature between male and female in the light or dark phases (Fig. 3A-D). Qualitative observations showed spikes of increased locomotor activity during the dark phase for both male and female groups, but no significance was observed. (Fig 3A-B). A circadian light/dark pattern was statistically significant for both the body temperature of male and female rats (male: $p = 0.001$, female: $p < 0.001$) (Fig. 3D).

Locomotor activity changes in SCI, TBI and Control animals

SCI induces a reduction on locomotor activity during the first week post-injury

The automated RFID digital activity data from the automated home cage recording system (data plotted 24/7 for the 3 days post-injury; 6 animals/group), showed a significant reduction on the locomotor activity of SCI animals during the first week post-injury relative to TBI ($p = 0.045$) and control animals ($p = 0.045$) (Fig. 4 & 5A). Interestingly, at week 6 post injury, there was a significant increase on the locomotor activity of SCI animals compared to TBI ($p = 0.026$) and control animals ($p = 0.039$) (Fig. 4 & 5B). However, at week 12 post injury, no significance between the groups were observed (Fig. 5C). When the light and dark phases were analysed, all CNS injury groups

293 showed a significant difference in locomotor activity at weeks 1, 6 and 12 post injury (Fig.
294 5A-C). Temporal analysis of the locomotor activity exhibited a significant decrease in SCI
295 animals at week 1 and 12 compared to week 6 post injury in the light phase ($p = 0.013$
296 and $p = 0.039$, respectively)(Fig. 5D). Furthermore, locomotor activity for SCI in the dark
297 phase at week 1 was significantly decreased compared to both weeks 6 and 12 post injury
298 ($p = 0.003$) (Fig. 5D). Interestingly, locomotor activity was not altered in TBI group (Fig.
299 5E), but the non-CNS injured control group exhibited a significant decrease in locomotor
300 activity at week 12 compared to week 1 in the light phase ($p = 0.007$), and a significant
301 increase in locomotor activity at week 6 compared weeks 1 and 12 in the dark phase (p
302 $= 0.007$) (Fig. 5F).

303

304 *SCI and TBI induces a reduction in body temperature at light phase of week 6 and 12*
305 *post-injury*

306 Subcutaneous body temperature recordings from automated RFID data (data plotted 24/7
307 for the 3 days post-injury; 6 animals/group) showed no significant changes between the
308 CNS injury groups during week 1 and 12 post injury (Fig 6 & 7A, C). Interestingly, at week
309 6 post injury, body temperature was significantly altered between the light and dark phase
310 for SCI ($p = 0.029$) and TBI ($p = 0.018$), but not the control group (Fig. 7B). Temporal
311 analysis of body temperature in SCI and TBI group exhibited a significant reduction in
312 body temperature at weeks 6 and 12 at light phase and between light and dark phases
313 (Fig. 7D & E). In the non-CNS injured control group, no significant alteration in body
314 temperature were observed between light and dark phase and in any temporal manner
315 (Fig. 7F).

316

317 **Temporal changes in selected behavioural phenotype in SCI, TBI and Control**
318 **animals**

319 *Feeding and drinking behaviour did not change over time with CNS injury*

320 Using the combination of RFID and IR Video data at week 1, 6 and 12 post-injury, detail
321 analysis of recordings at the duration of time in which rats spent feeding and drinking
322 were carried out. This was a proxy measure of food consumption and water intake,
323 respectively. No significant changes in the expression of either feeding or drinking were
324 observed between weeks 1, 6 and 12 for SCI, TBI and non-CNS injured control animals
325 (Fig. 8A-F). These data suggest that the CNS injuries in these animals do not significantly
326 limit the animals' ability to feed and drink *ad libitum*.

327

328 *Grooming behaviour was lowest in SCI rats in the first week after CNS injury*

329 The duration of time rats spent in individual grooming, by manual curation of the RFID
330 and IR Video data at weeks 1, 6 and 12 post-injury, as proxy measure of self-maintenance
331 were manually analysed. At week 1 post-injury, mean dark phase grooming was
332 significantly lower in the SCI than the non-CNS injured control group ($p = 0.044$) (Fig.
333 8G). Additionally, a trend difference ($p = 0.063$) in dark phase grooming behaviour was
334 also shown in TBI vs. SCI animals (Fig. 8G). However, thereafter at weeks 6 and 12, no
335 significance difference in grooming for any groups were observed (Fig. 8H-I). These data
336 suggest SCI interferes with the animals' grooming activity during the first week post injury.

337

338 *Rearing behaviour activity increases over time after SCI*

339 Using the RFID and IR video data, we manually analysed the duration of time rats spent
340 rearing as a proxy measure of hind limb motor function and possibly higher interest (e.g.
341 exploration, information gathering). Not surprisingly, at week 1 post injury, SCI animal
342 with hindlimb paralysis had significantly fewer rearing than the control animals at the dark
343 phase ($p = 0.037$) (Fig. 8J). No significance was observed in mean duration of rearing at
344 weeks 6 and 12, between the SCI, TBI and non-CNS injured control animals (Fig. 8K-L).
345 Temporal rearing activity from week 1 to week 12 did not exhibit any significant difference
346 within the non-CNS injured control or the TBI group (Fig. 9A & C). However, during the
347 dark phase, SCI animals exhibited a significant increase rearing in week 12 when
348 compared to week 1 ($p = 0.012$) (Fig. 9B). These data suggest SCI limits the animals from
349 carrying out rearing activity during the first week post injury.

350

351 *Aggression was significantly higher in TBI animals early after injury*

352 Using the RFID and IR Video data we manually recorded the duration of time that rats
353 demonstrated aggression, as a proxy measure of antagonism. In week 1 post injury, the
354 mean duration of aggressive behaviour was significantly higher in the TBI than SCI or
355 non-CNS injured control groups, during the dark phase ($p < 0.001$, $p = 0.004$,
356 respectively)(Fig. 9D). Also, aggression in TBI was higher in the dark phase than light
357 phase at 1 week post injury ($p < 0.001$) (Fig. 9D). At week 6 post injury, dark phase
358 aggression in the TBI group was also significantly higher than SCI group, and higher than
359 in the light phase ($p = 0.014$, $p = <0.001$, respectively) (Fig. 9E). At week 12 post injury,
360 there was no significant difference in the expression of aggression between groups (Fig.

361 9F). Temporal aggression activity in the TBI group was significantly higher at dark phase
362 week 1 post injury compared to light phase week 1 and dark phase week 6 and 12 (p
363 <0.001 , $p = 0.089$, $p <0.001$, respectively)(Fig. 9G). Interestingly, the aggression activity
364 was also significantly higher at week 6 post injury in dark phase compared to the light
365 phase ($p = 0.009$) (Fig. 9G). These data would suggest TBI have an acute increase in
366 aggression, which decreases with time at both light and dark phases.

367

368 **Assessment of injury severity**

369 *Behavioural assessment*

370 The BBB scores were measured daily during week 1 post injury, and then weekly up to
371 12 weeks post-injury in SCI and non-CNS injured control animals. Baseline pre-surgery
372 scores in both groups consisted of a BBB score of 21 (no functional impairments). The
373 scores were sharply reduced in SCI animals immediately after surgery (values <4 during
374 the first week post-injury) indicating limited hindlimb movements following CNS injury (Fig
375 9A). A subsequent gradual improvement was observed from week 3, reaching a plateau
376 by week 7 post-injury. Non-CNS injured control animals showed no functional impairment
377 after surgery, displaying baseline scores of 21 over the 12 weeks (Fig. 10A).

378 mNSS scores were measured in TBI animals for 3 days post injury. Following a normal
379 baseline average score of 0 points one day prior to surgery, a mild functional deficit (2/20
380 score) was detected on the first day post-injury, as expected for a mild “closed head” TBI
381 model. (Fig. 10B).

382 Correlation tests showed a significant strong association between the automated RFID
383 activity data recorded during the dark phases in the SCI groups and their BBB scores
384 ($R=0.08806$; $P<0.001$) and a relatively good correlation during the light phases activity
385 data recordings ($R=0.5169$; $P=0.001$) (Fig. 11A). (do we need to add per 5 min in the
386 legend of the graph ?

387

388 *Histological assessment*

389 Gross histological analysis in the SCI group revealed elongated partial thickness spinal
390 cord lesions, with significantly larger areas of cavitation associated with loss of CNS
391 tissue surrounded by disordered tissue extending away from the lesion (Fig. 10C & E).
392 The non-CNS injured control group exhibited no histological damage in the spinal cord
393 (Fig. 10C & E).

394 Gross histological analysis in the TBI group revealed no significant morphological
395 changes in tissue between contused brain and age-matched control brains (Fig 10D).
396 However, a significant enlargement of the ventricles was observed when compared to the
397 control group (Fig. 10D & F).

398 Correlation tests showed a strong association between the automated RFID activity data
399 recorded during the dark phase and the cord cavity size ($R=0.9755$, $P=0.0123$; Fig.11C),
400 in accordance with the correlation observed between BBB and cord cavity size
401 ($R=0.9297$, $P=0.0358$; Fig.11B) in the SCI group. However this correlation was moderate
402 when associated with the recorded activity during the light phase ($R=0.7234$. $p=0.1495$;
403 Fig 11C).

404 Correlations between the automated RFID activity data recorded during both the dark and
405 the light phases and the ventricle size in the TBI group were moderate ($R=0.5793$,
406 $P=0.5261$ and $R=0.5609$, $P=0.2511$; respectively) (Fig. 11E), similar to that for the mNSS
407 and the ventricle size ($R=0.8137$; $P=0.09$; Fig. 11D).

408

409 Discussion

410 The present study reports the ability to monitor spontaneous behavioural phenotype of
411 rat models of SCI and TBI grouped-housed in their home cages during 12 weeks post-
412 injury using an automated recording system. Distinct changes in phenotype within each
413 injury group at specific time points after injury, and also differences between the injury
414 groups were identified. SCI animals exhibited less locomotor activity during the acute
415 period following injury. TBI animals exhibited heightened aggressive behaviour during the
416 acute and mid-term period after injury. The automated home cage recording system
417 successfully enabled the continuous acquisition of individual behavioural and
418 temperature data from group-housed SCI, TBI and control rats, in their home cage
419 environment. Such home cage approaches have great potential to improving the
420 relevance of behavioural testing in such complex CNS injury models, facilitating long
421 term, non-invasive, non-task driven assessment, and with minimal environmental
422 interference.

423 Our findings also suggest that SCI has a significant impact on the animals' behaviour.
424 Significant reductions in their locomotor and rearing activities were expected due to
425 hindlimb paralysis, but its impact on grooming care was a novel observation. Undertaking

behavioural testing during the early phases post-injury can be challenging as it is likely to have more confounders associated to interventions such as surgery, anaesthesia and analgesia. But locomotor function tests are likely to show more significance during early injury times than later ones when the SCI animals have already started to regain locomotor and homeostasis functions and when improvements may be more difficult to assess. Therefore, assessing the spontaneous behaviour of SCI animals within their home cage environment provides a great source of very valuable new information with great potential for assessing the impact of any possible therapeutic approach on non-locomotor related behaviours in SCI animals. It also highlights the importance of providing a good care and welfare monitoring protocols supporting grouped housing conditions to enhance as much as possible the animals' natural behaviour, particularly within the early acute phase post-injury.

In this study, grooming activity was reduced in SCI animals, particularly during the first week post-injury, when compared to non-CNS injured control animals, possibly associated to injury-linked mechanical impairments. By week 6 and 12, SCI animals showed an improvement in grooming. It is important to note that our more detailed observations were carried out in time frames expressing high frequency of activity occurrence, thus we may be missing in grooming activity during more stationary behaviour periods. Alterations in grooming behaviour have been repeatedly studied in rat SCI, but mostly associated to the biomechanical impairments to groom effectively as a behavioural test, mostly in cervical SCI models (28-30). Grooming is also associated to the animal's self-care routine, and its failure may also be associated with mood impairments, such as depression caused by boredom and lack of social interaction (31).

449 Self-neglect and poor care has been reported in depressed SCI patients (32) and
450 similarly, SCI injury has also been associated with the animal's depressed state (33). Our
451 study provides an objective tool to investigate such socially associated cognitive
452 impairments in grouped animals and through long term recordings. It is quite likely that
453 prolonged immobility in SCI animals might affect their mood, triggering self-neglect and
454 diminishing self-grooming behaviour, similar to that seen in humans with SCI (34).

455 Rearing activity was also reduced in SCI animals during the first week post injury,
456 compared to week 12 post injury when spontaneous recovery in hindlimb functions have
457 occurred. Rearing is irrefutably dependent upon hind limb function and thus linked to BBB
458 scorings, and SCI animals have been shown to progressively regain function by 2-3
459 weeks post-injury. Functional CNS deficits may improve by local neuroplastic changes
460 (35) and also gradual strengthening of local signaling networks such as central pattern
461 generators, as previously suggested in SCI models (36). Therefore, automated home
462 cage recording system may facilitate new avenues to assess the pace and extent of
463 recovering of rearing activity, and in particular, allow to investigate the role of housing
464 enrichment to stimulate regular exercise and its impact on regaining functionality.

465 One of the major concerns when monitoring SCI and TBI animals is the ability of the motor
466 and cognitively impaired animals to feed and drink. Our study demonstrated using our
467 injury paradigm that neither TBI nor SCI significantly influenced feeding or drinking
468 behaviour. Yet the ability of the injured animals to access food and water should not
469 undermine the importance of good care and welfare monitoring of these animals, as
470 maintenance of an appropriate schedule of feeding and drinking will also have a direct
471 impact on the functional recovery following SCI and TBI (37).

472 We were also able to identify changes in aggressive behaviour between TBI, SCI and
473 control animals. The data demonstrated an increase in night-time aggression at week 1
474 post injury in TBI animals, compared to SCI and control animals. Such increased
475 aggressive behaviour persisted by week 6 post-injury in TBI animals, but was no longer
476 detected by week 12, when compared to SCI animals. Such assessment were based on
477 individual behavioural patterns, as described in Fig. 2, and may associated to specific
478 alteration of social rank rather than equal degree of aggression patterns for each
479 individual animal. The effect of TBI on aggressive behaviour in rodent models has
480 previously been reported in mice, but there are no reports in rat TBI (38). Assessment of
481 aggression in laboratory rodents is intrinsically challenging, owing to the diversity of
482 behavioural patterns and its multidimensional causes, expressions and functions. Animal
483 studies on aggression tend to focus on the ethological relevance to survival; that is
484 aggression that promotes access to food, territorial homing, mating, offspring protection
485 or social rank. However, CNS injury may precipitate a pathological aggression that
486 challenges such ethologically driven adaptive behaviour (39)- it is such maladaptive
487 aggression that we have attempted to evaluate here. So the challenges are associated
488 with the interpretation of different tests used for aggression, which are generally based
489 on stimulating a defensive response, the lack of clear relationship between aggression,
490 fear or defensiveness and how to account for the inhibitory effects of fear on the
491 aggressive response. Furthermore, the lack of clear translation between categories of
492 animal and human aggression, as human aggression is directly linked to complex
493 societal perceptions (40). Most preclinical testing for aggression is carried out using the

494 tube dominance test (41), but it remains uncertain whether outcomes directly relate to
495 human aggression.

496 The high incidence of aggressive behaviour in TBI patients is a major health concern (42,
497 43). Translational strategies need to search for new avenues to understand and to
498 evaluate aggression behaviour in animal models. The aggression data provided in this
499 study, based on the individual observation of published behaviour patterns in housed-
500 grouped animals, provides a new approach to monitor such challenging behaviours in
501 SCI or TBI animals (44-46).

502 There are several challenges and limitations in this study. Firstly, our automated home
503 cage recording systems were installed in our standard rat housing room, with no specific
504 restrictions on access to the room by other staff. Therefore, there was no specific control
505 for external stimuli influencing rats' activity (e.g. general daily husbandry activities, access
506 to the room by other researchers). Our primary objective was to assess the feasibility of
507 using the automated recording system in our animal unit, while maintaining our regular
508 husbandry and our animal care procedures, and thus minimizing confounding effects due
509 to stress or other environmental effects associated to changing the housing conditions of
510 the tested animals. Yet, most of the disruptive periods could be easily identified by the IR
511 video recordings and it was easy to exclude them from analysis based on time recordings.
512 A possible solution would be to keep the automated recording system in a dedicated room
513 with access restriction.

514 Secondly, although the generation of the body temperature and number of transition data
515 take approximately 5 min to complete using the HCA software analysis, the revision of IR
516 video recording is very time consuming. It may require multiple annotations when

517 assessing individual behaviour in group animals (each footage was reviewed 3 times to
518 focus on each one of the 3 caged animals in each revision). However, by integrating the
519 RFID and the IR video recording data we were able to rapidly select specific time frames
520 associated with the automated RFID data. While this approach allowed us to investigate
521 the detailed behaviours, including grooming, drinking, eating, aggression and rearing, our
522 combine analysis was focused on the periods of high activity for each cage individually
523 (expressed by ≥ 1 animal within the $n=3$ animals housed per cage; see Suppl. Fig. S2)
524 and that this was not the same time point for each cage. Therefore, each cage was
525 analysed at different times of the day, rather than a continuous assessment of each
526 individual animal per group. Data storage and handling could also be an issue, and it is
527 mostly associated with the storage of the IR video recording data due to the large data
528 files. However, as mentioned above, using the RFID automated data allows for a rapid
529 selection of specific time frames of video recording, improving data storage and
530 management.

531 Finally, we decided to use female rats for SCI studies and male rats for TBI studies as
532 these are the most commonly used sexes for the models. Females rats are often preferred
533 as easier to support bladder dysfunctions whilst male rats are driven by male TBI
534 prevalence. Although our preliminary studies on naïve animals (non-injured, $n=3$ males
535 and $n=3$ females, showed no differences on baselines activity and/or body temperatures
536 (Fig. 3), it is possible beyond baseline data that we cannot exclude differential responses
537 to CNS injury based on sex related endocrine effects.

538

539 *Impact on animal care and welfare and future perspectives*

540 The use of automated home cage analysis system has provided a unique opportunity for
541 evaluating the spontaneous behaviour in individual grouped-housed SCI and TBI animals
542 for a long 3 month period after injury. The system has facilitated the identification of novel
543 behaviour insights in SCI and TBI rat models, such as transient increase in aggression
544 following TBI, a transient reduction in grooming and rearing activity following SCI, and no
545 effect of either TBI or SCI on drinking or feeding patterns. The provision of such a unique
546 source of behavioural observations in SCI, TBI and control group-housed animals,
547 acquired in their own environment and with minimal interference, represents a major
548 improvement in the quality, quantity and scientific value of the experimental data
549 generated per animal. The monitoring versatility of this automated system to assess
550 cognitive /social behaviour in grouped animals compared to conventional out-of-cage
551 tests carried out in single isolated animals may enables complementary avenues to
552 identify socially-dependent behaviours that may be favourable or adversely affected by
553 treatment intervention. This along with the ability to support long term studies, with 24/7
554 recordings, may impact on the number of animals required for experimentation. Moreover,
555 being able to continuously and accurately monitor behaviour and body temperature has
556 significant implications for laboratory animal welfare; it can inform refinement of care and
557 monitoring protocols, severity limits and humane endpoints (17), which is particularly
558 pertinent for neurotrauma models. For instance, we report considerable impairments of
559 locomotion and thermoregulation in SCI animals during the initial weeks post-surgery,
560 which should translate in improved monitoring and care protocols. The ability to support
561 such non-invasive long-term behaviour assessments in complex injury models while
562 maintaining the animals housed in their own environment and cohorts represents an

563 important experimental refinement (in accordance with the 3Rs) and, by providing a
564 valuable complementary approach to other conventional tests, may overall strengthen
565 our understanding of the behaviour outcomes.

566 This technology considerably complements the accurate detection of subtle changes in
567 behaviour phenotype of these complex CNS injury models. For example, handler-
568 directed, compensatory aggression in response to removal from the home cage, for
569 running a tube dominance test, may render increases in baseline aggression secondary
570 to the neuroinjury undetectable (47). The automated analysis system provides an
571 accurate comprehensive platform for investigating a wide range of behaviours, free of
572 experimenter and environment interference. In summary, this technology represents a
573 major advancement on current methods for studying behaviour in neurotrauma models,
574 with great potential to enhance translational power of preclinical neurotrauma studies.
575 This warrants its application in further neurotrauma and drug discovery research, in order
576 to aid the development of effective new treatments for SCI and TBI.

577

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584 system in neurotrauma models.

585

586 Supporting information

587 **Supplementary Figure 1.** (A) The 21-point BBB (Basso, Beattie, Bresnahan) locomotor
588 scale (6) was used to assess the hind limb recovery in rats following thoracic SCI, based
589 upon observation of their spontaneous open-field locomotion. (B) The mNSS (modified
590 neurological severity score) for TBI in rats was used. It was modified from the original
591 score (48) to accommodate the mild nature of the closed head injury used in this study.

592

593 **Supplementary Figure 2:** Method of data sampling to assess in detail specific
594 behavioural expressions (e.g. aggression, grooming, rearing, feeding and drinking) by
595 reviewing the RFID digital data with the IR video recordings. (A) The objective was to
596 elucidate during a representative day of per group per week, when the rats were most
597 active and within that time frame which behaviours were being displayed. We selectively
598 reviewed the periods of maximum activity as we hypothesised that these periods should
599 show maximal expression of the stereotypical behaviours that characterise each
600 phenotype. Notably, we plotted 'transitions' against time because the HCA system/report
601 recorded 'transitions' as a proxy for 'activity'. Specifically, one transition defines the
602 movement of an animal's RFID chip across the electromagnetic field boundary between
603 two adjacent antennae. (B) A representative graph displaying the total number of
604 transitions. Yellow and grey shaded areas indicated the light and dark phases,
605 respectively. The arrows indicate the peaks with the greatest number of transitions
606 occurring within a 5 min interval, per 3 h division of each 'light or dark' phase that are not

607 caused by external events (e.g. person entering the room, changing water
608 bottle)(arrowheads).

609

610

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646 [analysis-and-](http://pilas.org.uk/automated-homecage-behavioural-analysis-and-the-implementation-of-the-three-rs-in-research-involving-mice) the-implementation-of-the-three-rs-in-research-involving-mice.

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728

729

730 **FIGURE LEGENDS**

731 **Figure 1. Overview of the experimental design.** After the baseline recording of the
732 behavioural tests, animals were subjected to CNS injury and implanted with the RFID chip
733 subcutaneously before they were returned to their group in their home standard IVC

734 cages. During automated analysis using the home cage analysis (HCA) unit, the ICV cage
735 were secured directly above the baseplate RFID reader to derive the positional and
736 temperature information for each individual animal from their RFID chip. An infrared HD
737 video camera captured an infrared gray scale video, supported by the illumination of an
738 array of infrared LEDs for the light/dark cycle recordings. RFID baseplate and video data
739 (24/7 h for 3 days/week for 12 weeks) was captured in a mini-computer. HCA units were
740 kept inside the rat housing room, to maintain environmental housing conditions.
741 Functional assessments were carried out daily for the TBI animals (mNSS scores; up to
742 3 days) and weekly for SCI and control animals (BBB scores; up to 12 weeks). 12 week
743 post-injury animals were humanely killed and tissue fixed-perfused for histology.

744

745 **Figure 2. Definitions of the five behavioural expressions selected and analysed in**
746 **detail in this study.** These were aggression, individual grooming, rearing, feeding and
747 drinking, with images directly acquired from the IR video recordings.

748

749 **Figure 3. Locomotor activity and body temperature of naïve rats.** (A) Data displays
750 the locomotor activity of the animals derived from the number of transitions detected by
751 the baseplate RFID reader from the individually ID chipped group-housed rats. (B) No
752 significant difference in locomotor activity was observed between naïve male and female
753 rats and light and dark phases. (C) Data displays the body temperature recording of the
754 animals measured through the subcutaneous chip in the lower flank of the animals. (D)
755 No significant difference in subcutaneous body temperature was observed between naïve
756 male and female rats, but significant difference was observed between light and dark

757 phases for both genders. Data plotted for male (blue) and female (red) rats over a 5 day
758 period from 24/7 recordings; mean \pm SEM of 3 rats per group. The 12 h light-dark phase
759 is indicated by white-black bars above graph.

760

761 **Figure 4. Locomotor activity in control, SCI and TBI animals at various weeks post**
762 **injury.** Data display the locomotor activity (number of transitions automatically detected
763 by the RFID reader) from the individually ID chipped group-housed rats. Representative
764 data plotted over 2 days per week 1 (A-C), week 6 (D-F), and week 12 (G-I) post surgery
765 from 24/7 recordings; mean \pm SEM per group. Note the lack of light/dark circadian
766 pattern in SCI and TBI animals during the first week post injury compared to the control
767 group. Furthermore, SCI group showed decreased activity patterns during the 1 week
768 post injury. The 12 h light-dark cycle is indicated by white-black bars above graph.

769

770 **Figure 5. Comparison of locomotor activity in control, SCI and TBI between weeks**
771 **post injury and injury groups.** (A) Significant decrease in locomotor activity in SCI group
772 compared to the control and TBI group at week 1 post injury. Significant increase in
773 locomotor activity observed in the dark phase compared to the light phase for all
774 groups.(B) Significant increase in locomotor activity in SCI group compared to the control
775 and TBI group at week 6 post injury. Significant increase in locomotor activity observed
776 in the dark phase compared to the light phase for all groups. (C) At week 12 post injury,
777 no difference between injury groups, but significant increase in locomotor activity in the
778 dark phase compared to the light phase for all groups were observed. (D) Temporal
779 changes in locomotor activity were observed in SCI animals within the light or dark phase.

780 (E) No temporal changes in locomotor activity was observed in TBI animals. (F) Temporal
781 changes in locomotor activity were observed in non-CNS injured control animals within
782 the light or dark phase. The 12 h light-dark cycle is indicated by white-black bar above
783 graph.

784

785 **Figure 6. Body temperature in control, SCI and TBI animals at various weeks post**
786 **injury.** Data display the subcutaneous body temperature (automatically detected by the
787 RFID reader) from the individually ID chipped group-housed rats. Representative data
788 plotted over 2 days per week 1 (A-C), week 6 (D-F), and week 12 (G-I) post surgery from
789 24/7 recordings; mean \pm SEM per group. Note the slower ability of SCI and non-CNS
790 injured control animals to recover normothermia immediately after surgery, compared to
791 the TBI groups even when warm post-surgery recovery chambers were used. Body
792 temperature levels did not show a circadian light/dark cycle pattern during the first week
793 post-surgery in all groups. The 12 h light-dark cycle is indicated by white-black bars above
794 graph.

795

796 **Figure 7. Comparison of body temperature in control, SCI and TBI between weeks**
797 **post injury and injury groups.** (A) No significant difference in body temperature
798 between the groups at week 1 post injury. (B) Significant decrease in body temperature
799 in SCI and TBI group at light phase compared to the dark phase at week 6 post injury.
800 Significant increase in locomotor activity observed in the dark phase compared to the light
801 phase for all groups. (C) At week 12 post injury, no significant difference between injury
802 groups. (D) Temporal changes in body temperature were observed in SCI animals within

803 the light and/or dark phase. (E) Temporal changes in body temperature was observed in
804 TBI animals within the light and/or dark phase. (F) No temporal changes in body
805 temperature were observed in non-CNS injured control animals. The 12 h light-dark cycle
806 is indicated by white-black bar above graph.

807

808 **Figure 8. Assessment of manually selected behavioural expressions for feeding,**
809 **drinking, grooming and rearing between control, SCI and TBI animals.** (A-C) No
810 significant difference was observed in feeding between all groups at week 1, 6 and 12
811 post injury. (D-F) No significant difference was observed in drinking between all groups
812 at week 1, 6 and 12 post injury. (G-I) SCI animals showed a decreased grooming activity
813 at week 1 during dark phase compared to control group ($P=0.04$), but by week 6 and 12,
814 no significant difference was observed between the groups. (J-L) No significant difference
815 was observed in rearing between all groups at week 1, 6 and 12 post injury. The
816 expression of a given behaviour was calculated as the duration of time (sec) that each
817 behaviour was performed by at least 1 animal within the cage during the 5 min period of
818 observation. Data presented as mean \pm SEM of 6 animal per group and during the light
819 and dark phases. The 12 h light-dark cycle is indicated by white-black bar above graph.

820

821 **Figure 9. Assessment of manually selected behavioural expressions for temporal**
822 **rearing within groups and aggression between and within groups.** (A) No significant
823 difference was observed in temporal rearing within the non-CNS injured control groups at
824 week 1, 6 and 12 post injury. (B) Significant difference was observed in rearing between
825 week 1 and week 12 at dark phase in SCI animals. (C) No significant difference was

826 observed in temporal rearing within the TBI groups at week 1, 6 and 12 post injury. The
827 expression of a given behaviour was calculated as the duration of time (sec) that each
828 behaviour was performed by at least 1 animal within the cage during the 5 min period of
829 observation. Data are presented as mean \pm SEM of 6 animals per group and during the
830 light and dark phases. White bars indicate LAM group, gray bars indicate SCI group, and
831 black bars indicate TBI group. The 12 h light-dark cycle is indicated by white-black bar
832 above graph.

833

834 **Figure 10. Assessment of injury severity using conventional behavioural and**
835 **histological analysis.** (A) BBB score of non-CNS injured control (blue square) and SCI
836 (black diamond) animals for 12 weeks post-surgery displayed severe initial hindlimb
837 impairment followed by some spontaneous functional improvement by week 6 post injury
838 compared to control animals. (B) mNSS score for the closed TBI injury (black circle) for 3
839 days post-surgery displayed limited functional impairment 24 h post compared to control
840 animals (blue square). (C) Representative Cresyl violet (Nissl) staining of serial horizontal
841 sections of spinal cord from control (left) and SCI (right) showing the degree of injury and
842 tissue damage across the whole spinal cord in SCI animals compared to the control
843 animals at 12 weeks post-surgery. (D) Representative Cresyl violet (Nissl) staining of
844 serial coronal sections of brain from closed TBI (right) displayed ventriculomegaly
845 compared to control brain (left) at 12 weeks post-surgery. (E) Analysis of the contused
846 spinal cord revealed significantly larger cavity than the control spinal cord. (F) Analysis
847 of the brain revealed significantly larger ventricles in the mild traumatic brain injury than

848 the control brain. * $p < 0.05$ and *** $p < 0.001$, Student's t test. Scale bars panel C, 0.5 mm
849 and panel D, 1 mm.

850

851 Figure 11. Correlation analysis between the automated RFID activity data and
852 conventional behaviour and histological tests. A) Activity data (nm of transitions) shows
853 highly significant good positive relationship with the BBB scores in the SCI group during
854 the dark phases and light phases of recordings across the 1, 6 and 12 weeks post-injury.
855 B) Good negative correlation between the BBB scores and cord cavity size (mm^2) in the
856 SCI group. C) Good negative relationship between the RFID activity data recorded during
857 dark phases and the cord cavity, and moderate relationship for the light phase data. D)
858 Moderate correlation between the mNSS and the ventricle size and E) the RFID activity
859 data recorded in the dark and light phases and the ventricle size in the TBI group. (should
860 you change the dark and light colour in the graph? Also the signs on Fig11A not too clear
861 on grey scale..)

862